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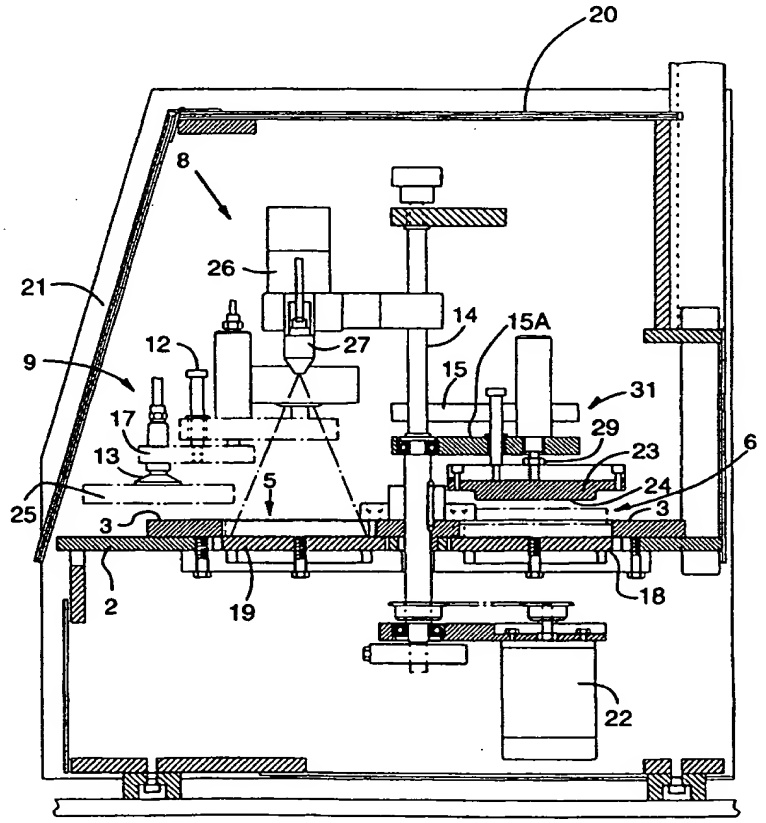
(54) Title: ROTARY THERMOCYCLING APPARATUS

(57) Abstract

A rotary thermocycling apparatus, especially for biochemical reactions. The apparatus comprises a plurality of and especially at least four stations for receiving biochemical samples in a flat-bottomed container. Each station has a flat heated plate on which the container is placed and means to independently control the heated plate at a pre-determined temperature. The apparatus has means to move each flat-bottomed container from one station to another station in a pre-determined sequence. At least two of the stations have a heating unit adapted to be lowered over a container located on the station, and at least one station has a spray unit adapted to spray liquid reagent(s) into a container located at that one station. A method is also described. The apparatus is used in, for example, polymerase chain reactions.



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TITLE

ROTARY THERMOCYCLING APPARATUS

The present invention relates to a rotary thermocycling apparatus, and to methods of using rotary thermocycling apparatus, especially for use in biochemical reactions and in particular for use in polymerase chain reactions (PCR). In embodiments, the present invention is directed to rotary thermocycling processes in which samples are placed on filters, or other media, and heated on plates in a sequence at predetermined temperatures, preferably including at least one step in which the sample is sprayed e.g. with liquid reagent(s), during each cycle.

The present invention will be particularly described herein with respect to biochemical reactions.

It is frequently necessary or desirable to be able to quantify and/or detect the presence of certain nucleic acid molecules or microorganisms in samples of air, soils, water, food, body fluids and other materials. This may be necessary in relation to an immediate medical or health situation, or in testing to determine safety for use by humans or animals. Thus, in many instances, it is important to be able to accurately and quickly confirm the presence and quantity of, or absence of, particular microorganisms in samples, and to do so in an automated, reliable and reproducible manner.

Traditional quantitative estimates of microorganisms in medical, food, environmental and other samples were based on colony counts after suitable culturing in diluted samples on nutrient agar plates. However, more accurate and rapid detection of microorganisms in various types of test samples has become possible.

Genetic information in all living organisms is carried largely in nucleic acids, either double-stranded deoxyribonucleic acids (DNA) or ribonucleic acid (RNA), and detection and discrimination on the basis of specific nucleic acid sequences has permitted the detection of the presence or absence of a

particular organism within a test sample. The development of the polymerase chain reaction (PCR) process for amplifying one or more targeted nucleic acid sequences within a sample has greatly facilitated processes for detecting and discriminating specific nucleic acid sequences, and hence specific organisms.

PCR methods of detection require multiple or cyclic chemical reactions to produce a desired product, under carefully controlled temperature conditions to ensure accuracy and reproducibility, in order to produce sufficient material to enable detection of a microorganism in the sample, or indicate absence of the microorganism. Apparatus and methods have been developed which permit the accurate control of the temperature of reaction vessels in which such PCR amplification reactions may be performed. For example, there are a number of thermocyclers used for DNA amplification and sequencing in which one or more temperature controlled elements or "blocks" hold samples containing the reaction mixture, and the temperature of the block is varied over time. In other systems, a robotic arm is used to move mixtures from one block to another. These systems include features which allow the user to program temperatures or temperature profiles of the block over selected periods of time so that various processes e.g. DNA denaturing, annealing and extension, can be efficiently accomplished.

Polymerase chain reaction (PCR) is a technique involving multiple cycles that results in the geometric amplification of certain polynucleotide sequences each time a cycle is completed. The technique is now well known. One example of PCR involves denaturing a double-stranded polynucleotide, followed by annealing at least a pair of primer oligonucleotides to the resultant single-stranded polynucleotides. After the annealing step, an enzyme with polymerase activity catalyzes synthesis of a new polynucleotide strand that incorporates the primer oligonucleotide and uses the original denatured polynucleotide as a synthesis template to produce a new double-stranded polynucleotide molecule. This series of steps (denaturation, primer annealing, and primer extension) constitutes a PCR cycle. As cycles are

repeated, the amount of newly synthesized polynucleotide increases geometrically because the newly synthesized polynucleotides from an earlier cycle can serve as templates for synthesis in subsequent cycles. Primer oligonucleotides are typically selected in pairs that can anneal to opposite strands of a given double-stranded polynucleotide sequence so that the region between the two annealing sites is amplified.

The temperature of the reaction mixture must be varied during each PCR cycle, and consequently varied many times during a test. For example, denaturation of DNA typically takes place at about 90°-100°C, annealing a primer to the denatured DNA is typically performed at about 40°-60°C., and the step of extending the annealed primers with a thermostable DNA-polymerase is typically performed at about 70°-75°C. Each of these steps may have an optimal temperature.

Examples of PCR are disclosed in US 4,683,202, US 4,965,188 and US 5,038,852.

Apparatus in which a temperature gradient is generated across a gradient block is described in US 5,525,300. Multiple reaction mixtures may be held in wells on the gradient block. In preferred embodiments, the gradient block is integrated into a thermocycler used for nucleic amplification reactions.

US 4,981,801 describes an apparatus for carrying out enzymatic cycling reactions including a turntable, a number of reaction vessels arranged in the turntable around the periphery and means to circulate antifreeze liquid through the reaction tank. Heaters and refrigerators are provided in order to obtain variations in temperature.

An apparatus to detect and enumerate a particulate analyte in a liquid sample comprising a filter element in a holder and means to heat and control the temperature of the filter element, is disclosed in WO 94/21780 of R.G.L. Wheatcroft and W.B. Berndt.

A method for detection and discrimination of multiple analytes using fluorescent technology is disclosed in US 5,723,294.

Additional apparatus and methods for conducting thermocycling reactions, especially polymerase chain reactions, in a rapid automated and controlled manner would be beneficial.

Accordingly, one aspect of the present invention provides rotary thermocycling apparatus, especially for biochemical reactions, comprising:

(a) a plurality of stations and especially at least four stations for receiving samples in a flat-bottomed container, each station having a flat heated plate on which said container is placed and having means to independently control said heated plate at a pre-determined temperature;

(b) means to move each said flat-bottomed container from one station to another station in a pre-determined sequence;

(c) at least two of said stations having a heating unit adapted to be lowered over a container located on said station; and

(d) at least one station having a spray unit adapted to spray liquid reagent(s) into a container located at said one station.

In a preferred embodiment of the invention, said station of (d) is adapted for removal of a cover plate from said container prior to activation of the spray, and for replacement of the cover plate after said spray has terminated.

In another embodiment, the container is a flat-bottomed container of a dimension less than that of the station.

In a further embodiment, the heating is comprised of a section with a flat lower surface that is adapted to be lowered into said container close to but not in contact with the sample in said container, especially into contact with a cover plate therein.

In a still further embodiment, the apparatus has a programmer for controlling at least (i) the temperature at each station, (ii) the dwell time in each station, (iii) the duration and timing of the spray, (iv) the number of sequential cycles for the biochemical reaction.

In another embodiment, the container is adapted to receive a filter having the sample thereon, and to receive a cover plate over said filter.

In yet another embodiment, the apparatus is programmable and automated.

In a further embodiment, the apparatus is adapted to process more than one sample at a time, up to one for each station in the apparatus.

In another embodiment, the heating units are on pistons that are lowered into the containers.

Another aspect of the present invention provides a method for a sequential reaction, especially a sequential biochemical reaction, at different temperatures, comprising:

(a) placing a sample in a flat-bottomed container;

(b) sequentially cycling said sample through predetermined changes in temperature by placing said flat-bottomed container on flat heated plate at each said temperature for a predetermined period of time;

(c) optionally spraying said sample with at least one liquid reagent;

(d) controlling at least (i) the temperature at each station, (ii) the dwell time in each station, (iii) the duration and timing of the spray(s), and (iv) the number of sequential cycles for the reaction.

In a preferred embodiment of the method of the invention, a biochemical sample is located on a filter, membrane, microtitre container or microscope slide in said container, especially a filter.

In another embodiment, the method is programmable and automated.

In a still further embodiment, the sample is subjected to a pretreatment prior to the method for the sequential reaction.

In a further embodiment, a spacer is placed on the filter and a cover plate is placed on the spacer.

In a further embodiment, the reaction is a polymerase chain reaction.

In another embodiment, the reaction is for detection of specific DNA sequences.

In a further embodiment, the sample is subsequently subjected to a photochemical detection process, especially fluorescence, to detect product of the reaction, and in particular to electronic recording thereof e.g. using a

video camera.

A further aspect of the present invention provides rotary thermocycling apparatus especially for biochemical reactions, comprising:

(a) a plurality of stations for heating samples in a flat-bottomed container at predetermined temperatures;

(b) means to move each said flat-bottomed container from one station to another station in a pre-determined sequence; and

(c) at least one station having a spray unit adapted to spray liquid reagent(s) into a container located at said one station.

Another aspect of the present invention provides a method for a sequential biochemical reaction at different temperatures, comprising placing a biochemical sample on a filter and sequentially cycling said biochemical sample through predetermined changes in temperature by heating said filter on a sequence of flat heated plates for a predetermined period of time.

A further aspect of the invention provides a method for a sequential biochemical reaction at different temperatures, comprising placing a biochemical sample on a filter and sequentially cycling said biochemical sample through predetermined changes in temperature, at least one step in the sequence involving spraying the sample with liquid reagent(s).

The present invention is illustrated by the embodiments shown in the drawings, in which:

Fig. 1 is a schematic representation of a rotary thermocycling apparatus of the invention, in plan view;

Fig. 2 is a schematic representation of the rotary thermocycling apparatus of Fig. 1, as seen through A-A;

Fig. 3 is a schematic representation of a front view of the rotary thermocycling apparatus of Fig. 1;

Fig. 4 is a schematic representation of a cross-section of a spray unit; and

Fig. 5 is a schematic representation of a cross-section of a sample on a filter within a container.

The present invention is described herein with reference to a rotary thermocycling apparatus having four stations, each at a pre-determined temperature. This is the preferred number of stations, but is understood that the rotary thermocycling apparatus could have more or fewer than four stations at pre-determined temperatures, depending on the particular use intended for the apparatus. It is understood that, if there were more than four stations, more than one station could have a spray unit.

For convenience, the container used in the apparatus will be generally referred to as a dish. However, other examples of containers are disclosed herein.

Fig. 1 shows a rotary thermocycling apparatus, generally indicated by 1. Rotary thermocycling apparatus 1 has floor 2 on which is located rotating table 3. Rotating table 3 has four stations viz. first station 4, second station 5, third station 6 and fourth station 7. Rotary table 3 rotates about shaft 14, so that a sample is moved in sequence from one station to the next. It is understood that the sample would be suitably located in a dish or other carrier receptacle, which would contact with the heated plate at each station.

Each location of the stations has a heated plate with each heated plate having means to independently control the temperature thereof at a pre-determined temperature. The four stations are located symmetrically around rotating table 3 i.e. at 90° intervals. The heated plates are located at fixed locations in floor 2. First station 4 has heated plate 10, second station 5 has heated plate 11, third station 6 has heated plate 18 and fourth station 7 has heated plate 19. The heated plates do not rotate with rotary table 3, but rather rotary table 3 rotates so that each station is located over a heated plate when rotary table 3 is in each position of the sequence. Rotary table 3 does not rotate continuously, but moves in steps with a dwell time at each step.

First station 4 is an open station i.e. it is open for the placement of sample on the station, and does not have any spray or heating unit associated with it, as occurs for the other three stations, as discussed herein.

Second station 5 has spray unit 8 located above the station. Spray

unit 8 is described in greater detail with respect to the subsequent drawings. Lid lifter 9 is located adjacent to second station 5. Lid lifter 9 has lid lifter shaft 17 which pivots about lid lifter pivot 12. The end of the lid lifter shaft 17 opposed to lid lifter pivot 12 has lid lifter suction cup 13 located thereon. Lid lifter 9 is adapted to rotate about lid lifter pivot 12 so that lid lifter suction cup 13 is located above second station 5. In addition, lid lifter 9 is adapted to move downwards towards second station 5. The operation of the lid lifter is discussed below.

Each of third station 6 and fourth station 7 has a heating unit associated therewith and which is lowered onto samples at these stations, as discussed herein. The heating units are not shown in Fig. 1 (see Figs. 2 and 3), but are attached to supports 28 and 29, respectively.

Each of the four stations has a heated plate located at the position of the station, as discussed above, that may be controlled at a predetermined temperature. The heated plate is most preferably heated using electrical heating. While the temperature of each of the stations may be varied over a wide range, and controlled at such temperature, a typical arrangement of temperature of the heated plates at the four stations is first station 50°C, second station 50°C, third station 72°C and fourth station 96°C. Such temperatures are typical of temperatures for use with polymerase chain reactions, but the temperatures may be varied. The apparatus of the present invention preferably has suitable automated control systems, as discussed below.

Fig. 2 relates to the apparatus of Fig. 1, as seen through A-A. Fig. 2 shows rotary thermocycling apparatus 1 located within housing 20. Housing 20 has housing window 21 along the front side of the apparatus, for viewing of the thermocycling apparatus in use. Housing 20 has floor 2 located away from the base of housing 20, primarily for the purpose of convenience and for location of motors and other operating parts of the thermocycling apparatus beneath floor 2. Shaft 14 extends upwards through floor 2 at a substantially central location, and is connected to drive motor 22 to effect rotation of shaft

14. Rotating table 3 is rotated by means of shaft 14. Shaft 14 could be adapted to provide support, with suitable bearings, for each of the heating units of the third and fourth stations, and for spray unit 8. However, the heating units would normally be attached to supports 15 and 16.

Second station 5 and third station 6 are shown as being located in rotating table 3. Spray unit 8 is located above second station 5 and first heating unit 31 is located above third station 6. Second station 5 has heated plate 11, which is located in floor 2 and is not part of rotary table 3. Similarly, third station 6 has heated plate 18, also in floor 2 and not part of rotary table 3.

First heating unit 31 is shown in a partially lowered position, as represented by support 15 being in position 15A. First heating unit 31 has heating section 23 located on the underside thereof. It will be noted that heating section 23 is of a shape so that central region 24 of heating section 23 will enter into third station 6 while the upper portion of heating section 23 extends beyond the circumference of third station 6. It is intended that heating section 23 would lower to a position such that central region 24 of heating section 23 would enter into third station 6. The purpose of central region 24 entering into third station 6 will be discussed below.

Second heating unit 32 is located above fourth station 7 in the same manner as first heating unit 31 is located above third station 6.

Spray unit 8 is located above second station 5. Spray unit 8 has lid lifter 9 associated therewith. Lid lifter 9 has lid lifter suction cup 13 connected, through lid lifter shaft 17, to lid lifter pivot 12. Lid lifter 9 is intended to rotate about pivot 12 so that suction cup 13 becomes located above second station 5. In addition, lid lifter 9 is then adapted to cause suction cup 13 to descend into second station 5 for the purpose of lifting a cover, 25 (see 64 in Fig. 5), from a sample dish located within second station 5, as discussed below. Fig. 2 shows cover 25 located on suction cup 13, but in a position disposed away from second station 5, to permit a spray from spray unit 8 to be sprayed onto a sample in second station 5.

Spray unit 8 may be of a variety of designs, but is shown in the form of a spray container 26 having a spray nozzle 27. In this embodiment, it is intended that spray container 26 would be an aerosol container or a pump action sprayer, or other type of spraying containing the required liquid reagent(s) to be sprayed onto the sample. A preferred embodiment of spray unit 8 is shown in Fig. 4.

Fig. 3 shows a front view of rotary thermocycling apparatus 1. This view shows first station 4 and second station 5, with first heating unit 31 of third station 6 located behind second station 5, and second heating unit 32 of fourth station 7 located behind first station 4. Heating section 23 is associated with first heating station 31 and heating section 30 is associated with second heating station 32. Spray unit 8 is shown as located above second station 5. Lid lifter 9 is located beside second station 5.

Fig. 4 shows a cross section of one embodiment of a spray unit. The spray unit shown in Fig. 4 has a spray canister 40 that is connected to a spray head 41, part of which is spray nozzle 42. Spray canister 40 is located between pistons 43 and 44 and held in place by spray holder 45, which is connected to pistons 43 and 44. Pistons 43 and 44 are mounted on spray mount 46. Piston rod 47 extends from spray holder 45 and terminates in piston plate 48. It will be noted that spray nozzle 42 abuts piston plate 48. Spray 49 extends from spray nozzle 42 to contact sample 50. It will be appreciated that a variety of spray units could be used, examples of which are illustrated in Fig. 1 and 2 and in Fig. 4.

Fig. 5 shows a cross section of a sample in a dish. The dish, 60, contains salt pad 61, which is optional, as discussed herein. Filter 62 lies on salt pad 61 and is held in place and separated from cover 64 by spacer 63. Spacer 63 is conveniently in the form of an O-ring. It is to be noted that cover 64 could be in a variety of forms, including a cover insertable into a dish or onto a dish.

In use, a sample is placed on a suitable carrier for use in the apparatus. The carrier is most preferably a filter, with the sample being placed

on the filter by standard filtration techniques. For instance, if the particular material to be tested is a solution e.g. a water sample or other fluid, the filter could be placed in a standard flat-bottomed filtration funnel and the sample filtered to be retained on the filter. This would result in the cells of the particular species to be detected being scattered randomly on the filter, which would facilitate detection at a later stage. In alternative procedures, the sample could be on a membrane, a microscope slide or in a microtitre dish, or in some other suitable form. It is understood that the sample should be substantially planar and of a size that will fit within the dish that is used in the rotary thermocycling apparatus 1. It is understood that the dish referred to herein is any suitable flat bottomed receptacle that will fit within the stations of the apparatus and retain the sample, including cover plates, salt pads or any other item placed within the dish that is related to the reaction being conducted.

In order to conduct a polymerase chain reaction, the heating plates associated with the positions of the rotary thermocycling apparatus are heated to predetermined temperatures. For example, the first station could be at temperature of 50°C, the second station at the same temperature, third station at 72°C and fourth station 96°C, it being understood that the temperatures are optimized for particular types of reactions. The sample is conveniently placed in the first station. If two samples are to be tested at the same time, the unit would be manually turned and the second sample placed in the station opposed to the first station. If four samples are to be tested, the unit would be manually rotated and samples placed in each of the stations.

The rotary thermocycling apparatus would preferably have automatic controls to control temperatures, as well as control other parameters relating to the process, particularly the dwell time at each station, the duration and timing of any spray that is used and the number of sequential steps in the reaction.

The sample is placed in the rotary table at the first station and then rotated to the second station. At the second station, lid lifter 9 rotates over

the second station and lid lifter suction cap 13 is moved downwards to contact the cover plate on the sample. The suction cup 13 is then retracted and moved out of position, taking with it the cover, and thereby exposing the sample on the filter. Piston rod 47 of the spray unit (see Fig. 4) then retracts into piston 43, whereby piston plate 48 pushes on spray nozzle 42. This causes the spray to activate and liquid reagent(s) to be sprayed downwards onto the filter. The duration of the spray may be any convenient length of time, typically 0-5 -2 seconds, after which the procedure is reversed and the cover is replaced on the filter. At the appropriate cycle time, rotary table 3 rotates so that the sample becomes positioned at the third station. At this station, heating unit 31 descends onto the sample and into contact with the cover. The sample at this time is heated from below by the heated plate to a pre-determined temperature, for example 72°C, and simultaneously heated from above by the heating unit to the same temperature. At the end of the cycle time, rotary table 3 rotates to position the sample at the fourth station, at which it is heated from below by the heating plate at this station to a pre-determined temperature, for example 96°C, and from above by heating unit 32 to the same temperature. It is understood that the temperatures would be optimized for the particular reaction.

The sample is then rotated back to the first station, and is cooled to 50°C, the pre-determined temperature at that station. It is preferred that as the rotary table 3 rotates the sample from the fourth station to the first station, that the rotary table also effect a turning of the sample e.g. by 25-50°. This may conveniently be obtained by means of a ratchet mechanism on the side of the rotary table 3 (not shown). The turning of the sample to change the orientation of the sample in rotary table 3 randomises any inconsistencies in, in particular, the spray unit, as well as heating of the sample so that, after a number of cycles, all parts of the sample on the filter are treated to essentially the same conditions.

Accelerated cooling of the sample between the fourth station, which is at a temperature of for example 96°C, and the first station at a temperature of

for example 50°C may be desirable. Such accelerated cooling could involve use of refrigeration, air jets and/or fans between the fourth and first stations. A preferred method of cooling is use of a vortex tube, or a ranque vortex tube, to direct a stream of cooling air onto the sample. One example of a vortex tube is an EXAIR™ vortex from EXAIR Corporation of Cincinatti, Ohio, which is constructed of stainless steel.

The filter needs to be made from an inert material i.e. a material that is stable under the conditions of use, especially temperature, which does not affect the sample on the filter. A preferred filter is formed from nylon, with a pore size of 0.2 mm. If the dish that is used has a diameter of 92 mm, then the filter is conveniently of a diameter of 90 mm. The sample of bacteria, or other target, becomes trapped between the fibers of the filter (membrane).

Extraneous extracellular DNA would normally be removed from cells by incubation of the sample with a nuclease solution applied in a fine spray. The nuclease would then be inactivated and denatured by heat.

Bacterial cells would normally be lysed by a treatment suitable for the expected species. This could include one or more of the following: temperature shock, enzymic digestion and application of a detergent. Removal of extracellular DNA and lysing of bacterial cells would normally take place prior to placing the sample in the rotary thermocycler apparatus.

It is preferred that the dish contain a pad of salt, particularly an 89 mm diameter glass-fibre filter disc that contains the solutes required for the PCR process. The spacer is conveniently a silicone rubber O-ring, having an external diameter of 92 mm. The cover is preferably an 89 mm Teflon™ fluoropolymer cover, in the form of a flat disc.

Reference is made herein to the use of covers on the samples, which are removed prior to spraying with reagent and then replaced. A wide variety of covers could be used, including discs as described herein and lids that fit over the sample container (dish). Preferred covers fit closely to but are spaced from samples e.g. by use of O-rings, but also permit heating units to be lowered into contact therewith for purposes of rapid and easy temperature

adjustment and temperature control of the sample. All types of covers are generally referred to herein as cover plates but are to be understood to include discs and other types of covers.

In preferred embodiments of the invention, the spray unit is a small aerosol container or pump spray unit containing the liquid reagent(s) for the reaction. A small aerosol container of reagent(s) is convenient, as it enables the container to be readily replaced with another container of the same or different reagent(s). However, other types of spray units may be used with the apparatus of the invention.

Although the present invention is particularly described herein with reference to use of one spray unit, more than one spray unit could be used at the same or different stations. For instance, a programmed sequence of at least two different sprays could be used. It is also understood that in some circumstances, the spray(s) could be used in other than each cycle of the apparatus. More than one liquid reagent may be used, either as a combination of reagents in one spray unit, or as reagents in separate spray units that are sprayed simultaneously, in sequence in the same cycle, in different cycles or in some other manner.

The apparatus has been particularly described herein with respect to manual insertion and removal of the flat-bottom containers onto and from the first station. However, it is understood that flat-bottom containers could be automatically inserted onto and removed from the first station, to permit more efficient use of the apparatus and the ability of the apparatus to operate essentially unattended by an operator, including overnight, or for other reasons.

The apparatus has been described herein with a shaft 14, which could be used for support of heating units, spray units or the like. However, in a preferred embodiment (not shown), shaft 14 is omitted and other means are provided to support heating, spray and other units. Omission of shaft 14 permits rotating table 3 to be a removable table, to permit cleaning of the table and beneath the table and to permit replacement of the table with

another table e.g. with stations of different sizes or shapes e.g. square or oval shapes, or to expedite adaption of the apparatus to a different number of stations.

After use of the rotary thermocycler apparatus, it is necessary to take steps to detect the reaction products on the filter. In one example of such steps, double-stranded DNA reaction products on the filter may be sprayed with SYBR™ DX reagent from Molecular Probes, Inc. of Eugene, Oregon, USA., or another stain, specific for the double-stranded DNA. The stained clots of double-stranded DNA fluoresce as dots on a dark background under ultra-violet (UV) light. A photo image may be captured by an electronic camera and quantitatively analyzed using computer software to give a cell count for the targeted bacteria in the original sample.

Single-stranded DNA reaction products are tested for hybridization with a single-stranded DNA sequence probe specific to the expected product. Such probes may be readily detected using radio active labels, or labels that induce photochemical reactions e.g. chemiluminescence, or by other methods. This test would allow for authenticity of the PCR product to be checked simultaneously with quantitative analysis.

It will be understood that a wide variety of steps could be taken to detect, identify and quantify reaction products on the filter or other medium used.

The original sample of bacterial cell suspension or other material received for testing will frequently be diluted prior to filtering the sample. Thus, the count obtained at the end of a test e.g. using fluorescence, will need to be adjusted to reflect any dilution.

In some circumstances, it may be desirable to conduct one or more preliminary steps in preparation of a sample prior to conducting PCR or other tests in the apparatus of the invention. For instance, a sample of for example bacterial cells on a filter could be placed on growth medium agar and incubated. The cells would multiply, and the resultant micro colonies would be more readily detected using PCR in the apparatus described herein.

A mixed population of cells may be tested. In a mixed population, it is possible to detect one or more specific cell types containing the target DNA, so that the presence or absence of such cell types may be detected, while not detecting other cell types that do not contain the target DNA.

PCR specificity depends primarily on the choice of primers used to prime the target DNA synthesis and the stringency of the reaction conditions e.g. temperature of DNA annealing, concentration of reagents and other factors. This could enable the detection of a carrier of a particular gene, or solely those members of a taxonomic group that act as a carrier of a DNA sequence diagnostic for that group.

In use of the apparatus of the invention using a spray, DNA polymerase enzyme is sprayed on the sample during each cycle, or as otherwise programmed in the sequence of steps in the apparatus. Consequently, it may not be necessary to use a heat-stable enzyme that must be sufficiently stable to denaturation temperatures to be active during the entire PCR process. Even if the enzyme were to be fully or partially destroyed at the denaturation temperatures being used, it would be replaced in the next spray step. Thus, it might be beneficial to use a cheaper less heat-stable enzyme, and possibly more of such an enzyme, rather than a more expensive enzyme that would be stable at the denaturation temperatures used in each cycle. As an example, it might be possible to use Klenow enzyme in place of Taq DNA polymerase in a PCR process; with appropriate adjustment of the optimal temperature of use, and obtain acceptable results.

While the present invention has been particularly described herein with reference to PCR, other reactions could be carried out e.g. involving bacteria, DNA fragments in gel profile blots, viruses or the like, provided the samples could be obtained in a suitable form and appropriate occupational health precautions taken if necessary.

The heating units of stations five and six have an important effect on the rate at which the sample reaches the required temperature, by reducing

the depth of space that has to be heated. Thus, the volume to be heated extends from the cover to the base of the dish, which is simultaneously being heated from below. In the present invention, that volume is small.

An important aspect of the present invention is that the rate of cycling may be increased, because each station remains at its pre-selected temperature, and it is not necessary to change the temperature of a metal block frequently during each cycle.

The apparatus and method of the present invention may be used in a wide range of tests. Examples of such tests include detection of the presence of E. coli, Listeria and Salmonella, and other bacteria, using reagents known for use in detection of such bacteria. The apparatus and methods of the invention may also be used in other types of tests that require cycling of temperatures and spray of reagents. For instance, the apparatus and methods could be usable with some metallic catalyst reactions e.g. platinum catalysed reactions using hydrogen peroxide, other reactions that use thermocycling, formation of layered polymer structures in which thin layers e.g. of a thermosetting polymer, are polymerized in each cycle, and in detection techniques that require thermocycling.

CLAIMS:

1. A rotary thermocycling apparatus, especially for biochemical reactions, comprising:

(a) a plurality of stations and especially at least four stations for receiving samples in a flat-bottomed container, each station having a flat heated plate on which said container is placed and having means to independently control said heated plate at a pre-determined temperature;

(b) means to move each said flat-bottomed container from one station to another station in a pre-determined sequence;

(c) at least two of said stations having a heating unit adapted to be lowered over a container located on said station; and

(d) at least one station having a spray unit adapted to spray a liquid reagent(s) into a container located at said one station.

2. The apparatus of Claim 1 in which said station of (d) is adapted for removal of a cover plate from said container prior to activation of the spray, and for replacement of the cover plate after said spray has terminated.

3. The apparatus of Claim 1 or Claim 2 in which the container is a flat-bottomed container of a dimension less than that of the station.

4. The apparatus of any one of Claims 1-3 in which the heating unit is comprised of a section with a flat lower surface that is adapted to be lowered into said container close to but not in contact with the sample in said container.

5. The apparatus of Claim 4 in which said lower surface of the heating unit is lowered into contact with a cover plate therein.

6. The apparatus of any one of Claims 1-5 in which the apparatus

has a programmer for controlling at least (i) the temperature at each station, (ii) the dwell time in each station, (iii) the duration and timing of the spray(s), and (iv) the number of sequential cycles for the biochemical reaction.

7. The apparatus of any one of Claims 1-6 in which the container is adapted to receive a filter having the sample thereon, and to receive a cover plate over said filter.

8. The apparatus of any one of Claims 1-7 in which the apparatus is programmable and automated.

9. The apparatus of any one of Claims 1-8 in which the apparatus is adapted to process more than one sample at a time, up to one for each station in the apparatus.

10. The apparatus of any one of Claims 1-9 in which there is an automatic loader for placing containers into the apparatus and for removing containers therefrom.

11. The apparatus of any one of Claims 1-10 in which the heating units are on pistons that are lowered into the containers.

12. The apparatus of any one of Claims 1-11 in which the station with the spray unit is followed in sequence by the two stations having heating units.

13. A method for a sequential reaction especially a sequential biochemical reaction at different temperatures, comprising:

(a) placing a sample in a flat-bottomed container;

(b) sequentially cycling said sample through predetermined changes in temperature by placing said flat-bottomed container on flat heated

plates at each said temperature for a predetermined period of time;

(c) optionally spraying said sample with at least one liquid reagent;

(d) controlling at least (i) the temperature at each station, (ii) the dwell time in each station, (iii) the duration and timing of the spray(s), and (iv) the number of sequential cycles for the biochemical reaction.

14. The method of Claim 13 in which the biochemical sample is located on a filter, a membrane, a microtitre container or microscope slide in said container.

15. The method of Claim 13 or Claim 14 in which the method is programmable and automated.

16. The method of any one of Claims 13-15 in which the sample is subjected to a pre-treatment prior to the method for the sequential reaction.

17. The method of any one of Claims 13-16 in which a spacer is placed on the filter and a cover plate is placed on the spacer.

18. The method of any one of Claims 13-17 in which the reaction is a polymerase chain reaction.

19. The method of any one of Claims 13-17 in which the reaction is for detection of specific DNA sequences.

20. The method of any one of Claims 13-17 in which the reaction is for detection of bacteria.

21. The method of any one of Claims 13-17 in which the reaction is for enumeration of a distinct type of bacterium or species.

22. The method of any one of Claims 13-21 in which the sample is subsequently subjected to a photochemical detection process to detect product of the reaction.

23. The method of Claim 22 in which the photochemical detection process comprises fluorescence.

24. The method of Claim 22 or Claim 23 in which there is electronic recording thereof.

25. The method of Claim 24 in which the electronic recording comprises using a video camera.

26. A rotary thermocycling apparatus especially for biochemical reactions, comprising:

(a) a plurality of stations for heating biochemical samples in a flat-bottomed container at predetermined temperatures;

(b) means to move each said flat-bottomed container from one station to another station in a pre-determined sequence; and

(c) at least one station having a spray unit adapted to spray liquid reagent(s) into a container located at said one station.

27. A method for a sequential biochemical reaction at different temperatures, comprising placing a biochemical sample in a container and sequentially cycling said biochemical sample through predetermined changes in temperature by heating said container on a sequence of flat heated plates for a predetermined period of time.

28. A method for a sequential biochemical reaction at different temperatures, comprising placing a biochemical sample on a filter or

membrane and sequentially cycling said biochemical sample through predetermined changes in temperature, at least one step in the sequence involving spraying the sample with at least one liquid reagent.

29. The method of Claim 28 in which the sample is on a filter having an underpad of absorbed salts.

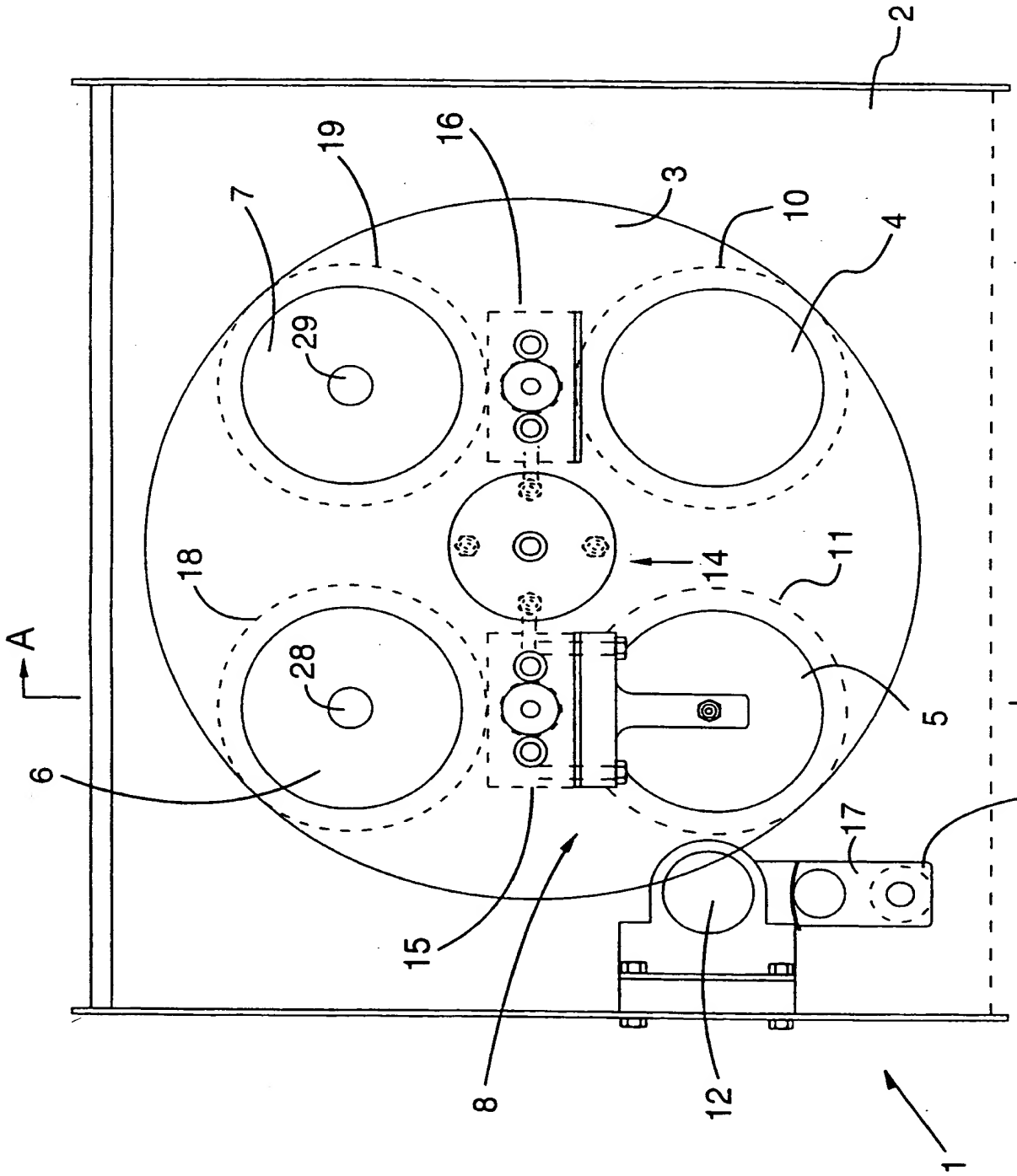

FIG.1

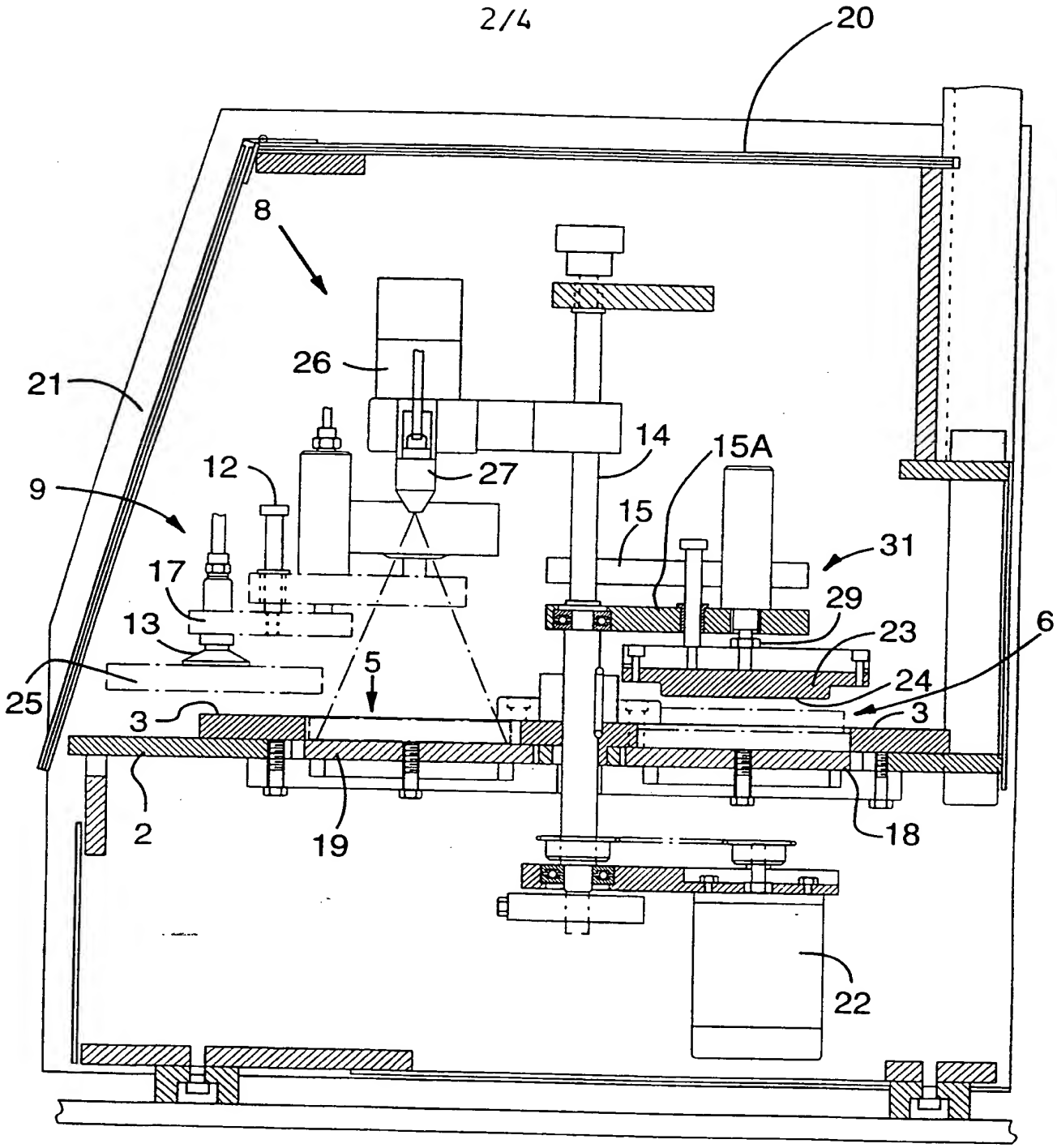

FIG.2

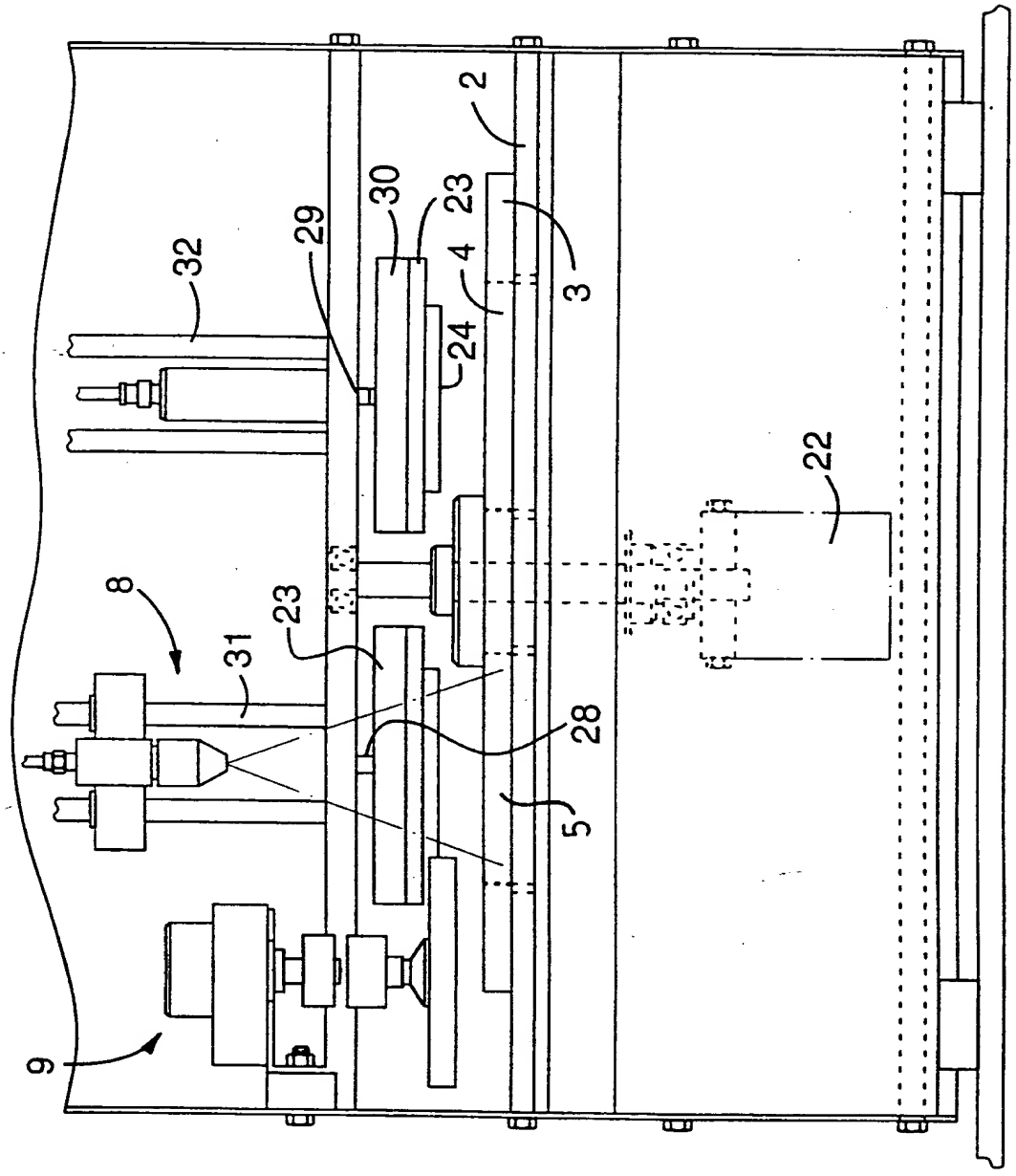

FIG.3

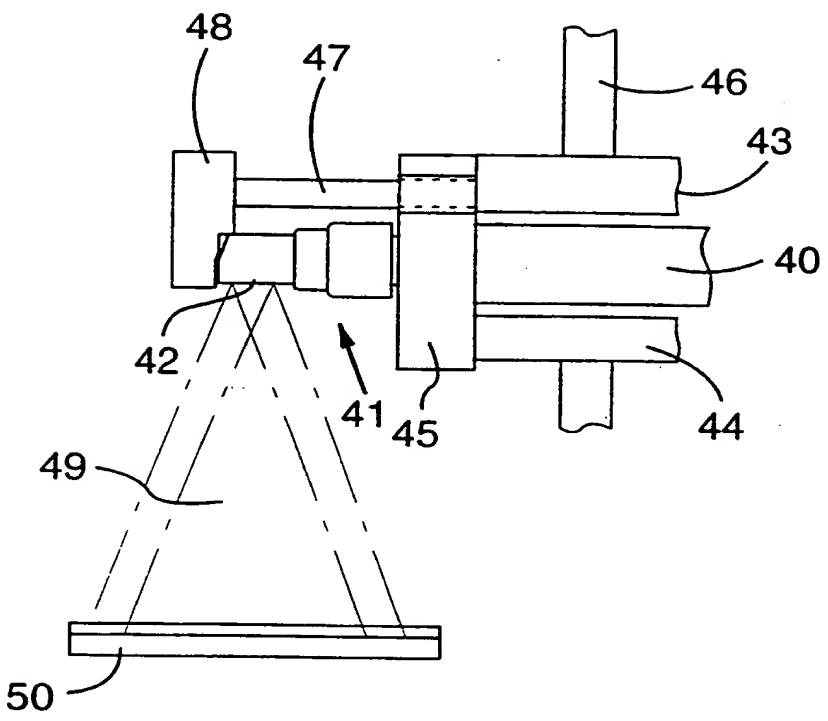

FIG.4

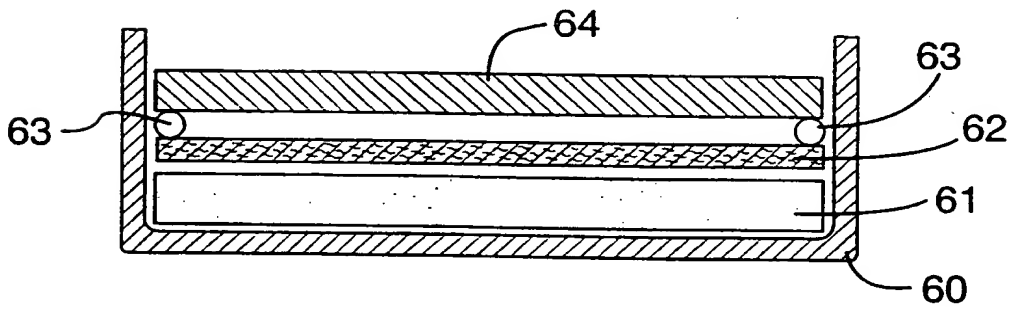

FIG.5

INTERNATIONAL SEARCH REPORT

Inter. nal Application No
PCT/CA 99/01035

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B01L7/00 //C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 B01L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4 981 801 A (SUZUKI YOSHIYUKI ET AL) 1 January 1991 (1991-01-01) cited in the application	1,3-6, 8-15,18, 19,22-25
X	column 11, line 18 -column 13, line 32; figures 7-10	26
Y	EP 0 723 812 A (TOSOH CORP) 31 July 1996 (1996-07-31)	1,3-6, 8-15,18, 19,22-25
X	column 4, line 21 -column 5, line 45	27
A	column 7, line 2 - line 10 column 7, line 15 - line 18 column 7, line 19 - line 23; figures	26,28
A	EP 0 438 883 A (BECKMAN INSTRUMENTS INC) 31 July 1991 (1991-07-31) column 5, line 22 -column 6, line 32	1,3-5,13
	-/—	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 February 2000	23/02/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hodson, M

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INTERNATIONAL SEARCH REPORT

Inter. nal Application No
PCT/CA 99/01035

C.(Continuation) DOCUMENTS CONSIDERED T BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
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A	DE 196 46 115 A (EPPENDORF GERAETEBAU NETHELER) 14 May 1998 (1998-05-14)	
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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter nal Application No
PCT/CA 99/01035

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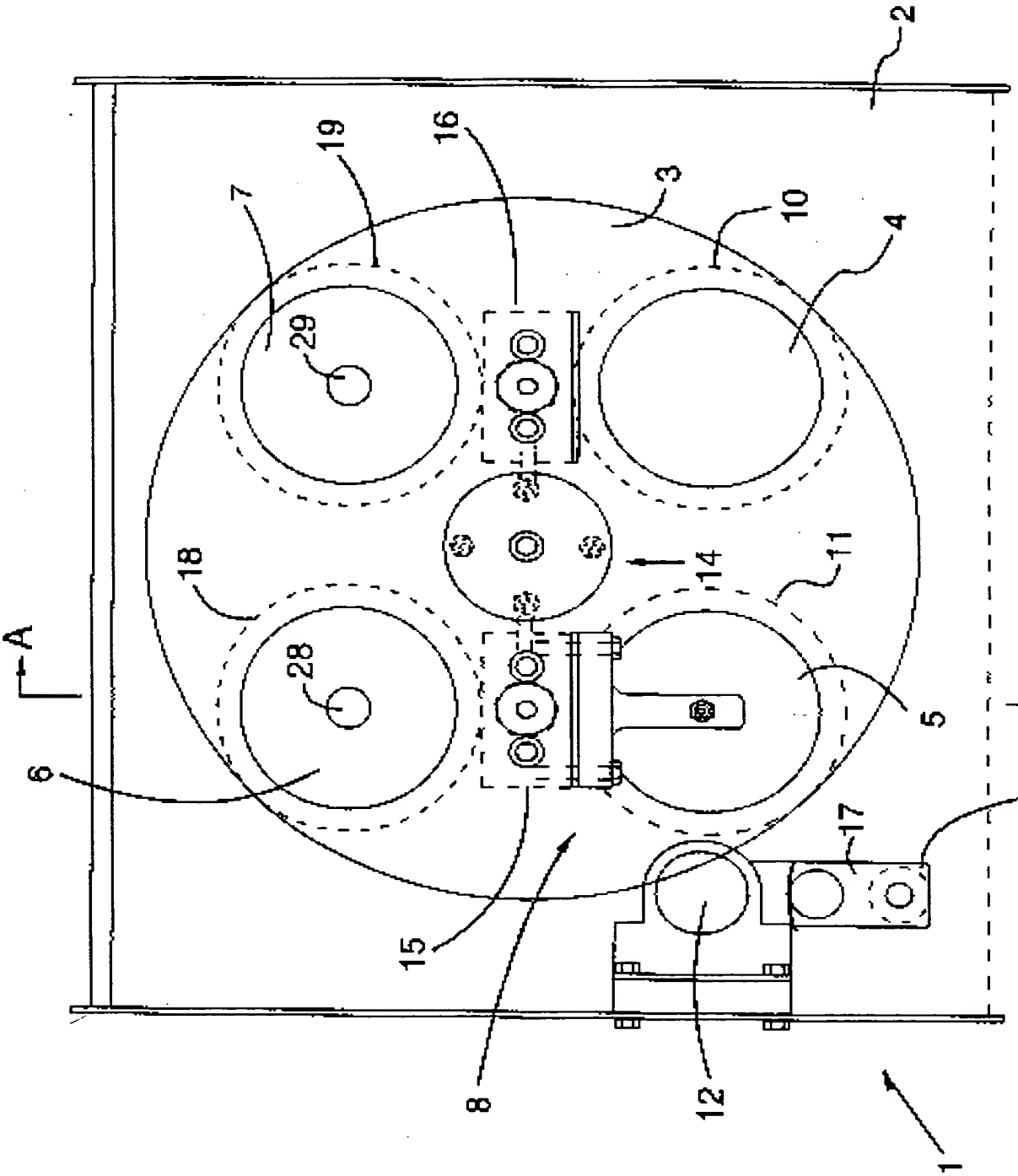

FIG.1

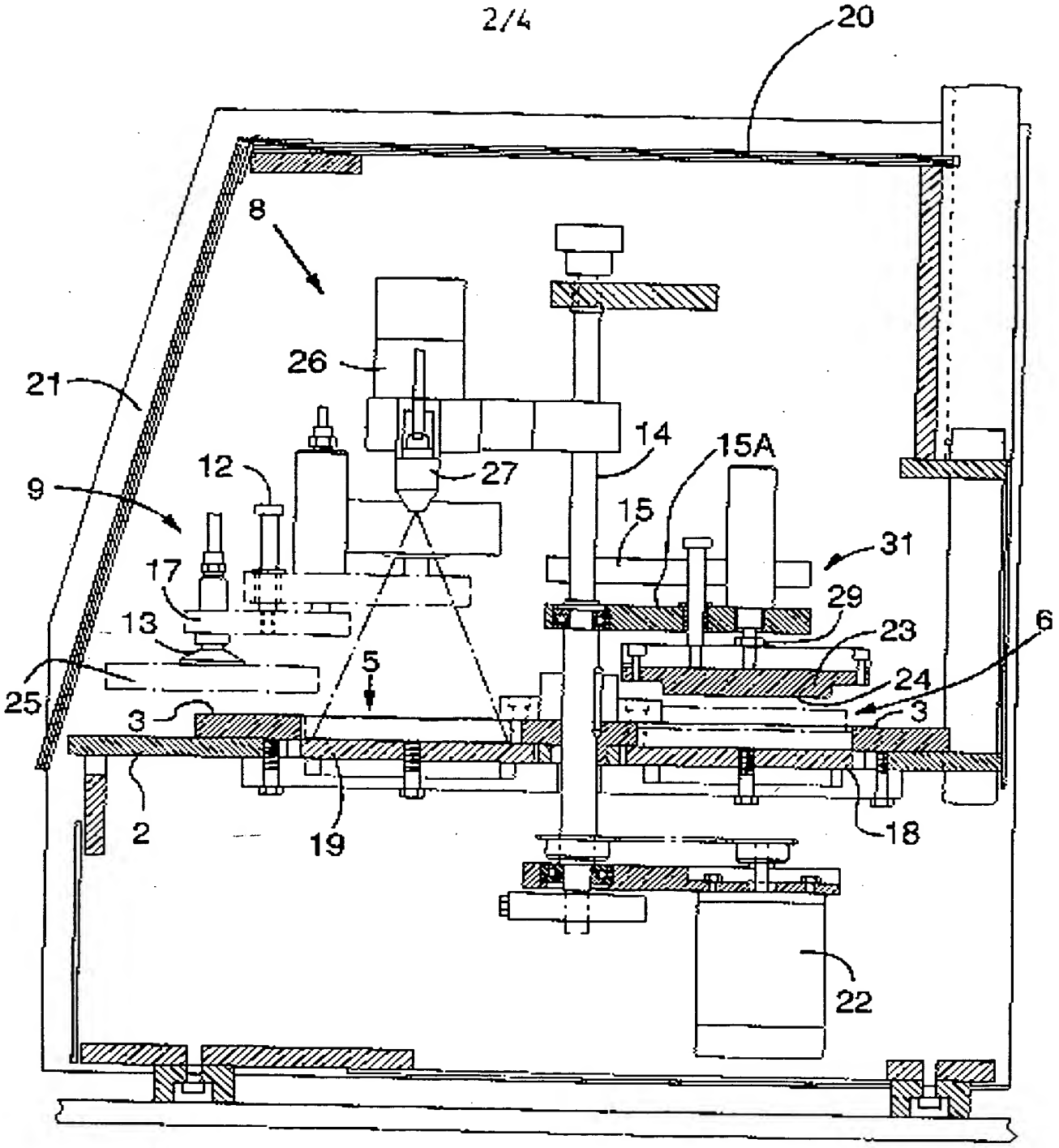

FIG.2

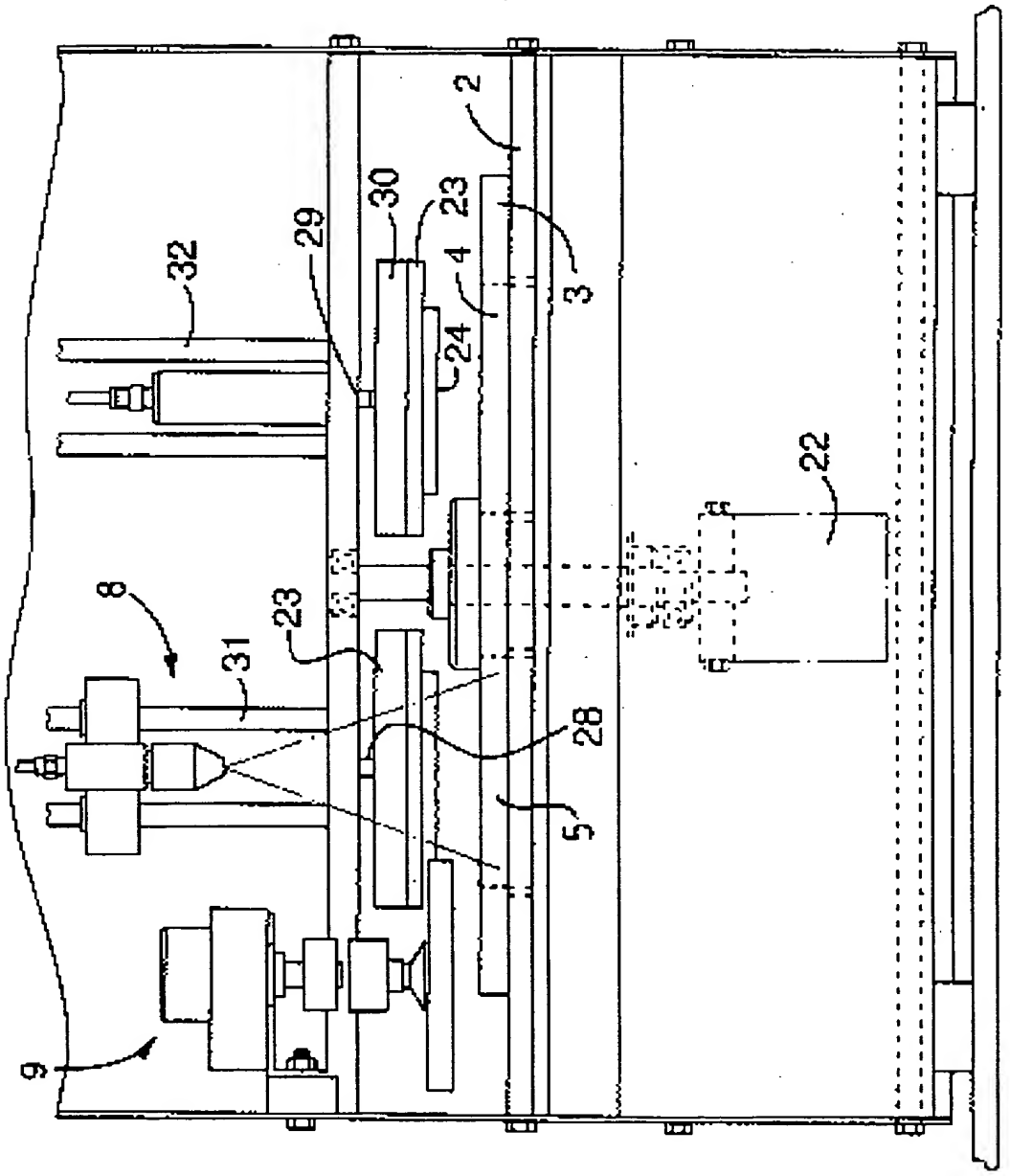

FIG.3

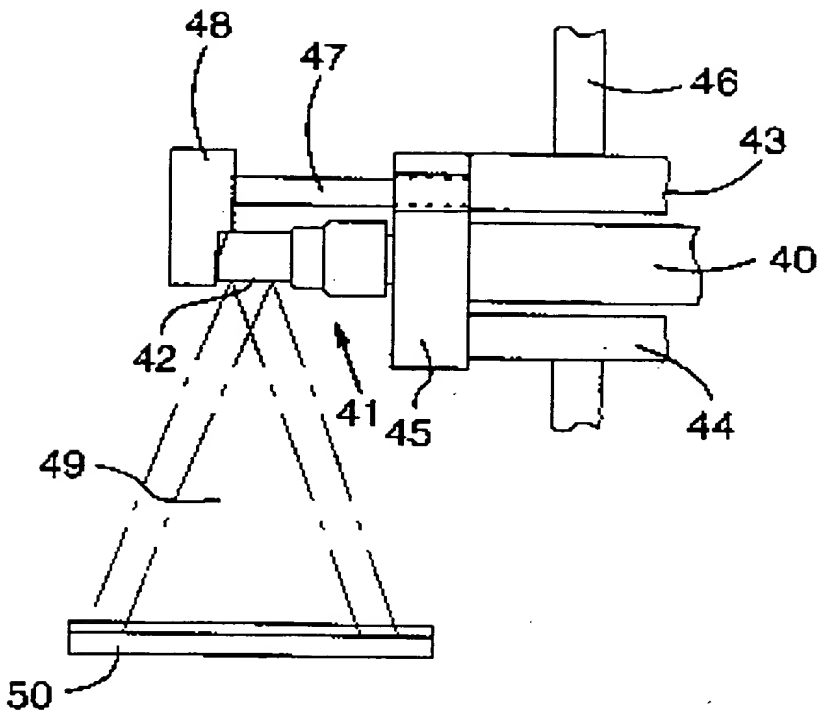

FIG.4

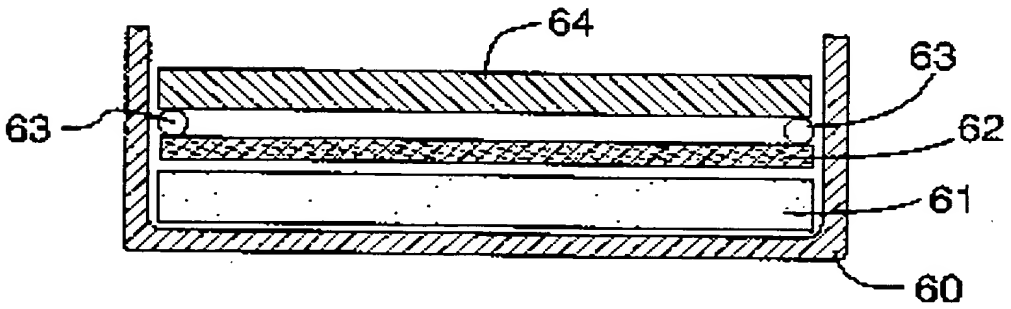

FIG.5